

UROGEN WELL D-ONE®

System for the presumptive identification and antimicrobial susceptibility of urogenital mycoplasmas, *Neisseria spp.*, *Streptococcus B*, *Staphylococcus aureus*, *Enterococcus spp.*, *Escherichia coli*, *Gardnerella vaginalis*, *Trichomonas vaginalis*, and *Candida spp.*

1 . INTRODUCTION

Mycoplasma hominis and *Ureaplasma urealyticum* / *parvum* are the species of the class Mollicutes isolated more frequently from the urogenital tract and considered opportunistic pathogens. It has demonstrated a direct relationship between the isolation of these microorganisms and certain diseases such as bacterial vaginosis (BV), pelvic inflammatory disease, infections during pregnancy , preterm labor and neonatal infections ⁽¹⁻²⁾

Rapid diagnosis available today may involve the use of expensive tests not available in all hospitals and not useful for determining antimicrobial susceptibility, essential for treatments implementations, epidemiological studies, etc.

In the light of this, a system that allows rapidly growing, in addition to antimicrobial susceptibility test in just 18-24 hours, without additional equipment, can be an useful tool in the hands of the microbiologist and clinician.

In many cases, the positivity of urogenital mycoplasma is associated with the presence of other microorganisms that cause genitourinary infections , such as *Neisseria spp.*, *Streptococcus agalactiae* (group B), *Staphylococcus aureus* , *Enterococcus spp.*, *Escherichia coli* , *Gardnerella vaginalis* , *Trichomonas vaginalis*, and *Candida spp* . The simultaneous identification of these agents can guide the clinical diagnosis and the procedure to follow with the patient. ⁽¹⁾⁽⁷⁾⁽⁸⁾

2 . PRINCIPLE

System composed of a polypropylene plate containing 32 conical wells for better viewing of the colorimetric reactions that occur as result of the growth of microorganisms in specially formulated media for selective culture of: *Mycoplasma hominis*, *Mycoplasma spp.*, *Ureaplasma urealyticum/parvum*.

The Kit allows the presumptive identification of *Neisseria spp.*, *Streptococcus agalactiae* (group B), *Staphylococcus aureus*, *Enterococcus spp.*, *Escherichia coli*, *Gardnerella vaginalis*, *Trichomonas vaginalis* and *Candida spp*. The system allows to perform, directly from the content of the wells, the sowing in selective media, the microscopic observation, the serological and molecular tests, etc. ⁽⁶⁾⁽¹¹⁾⁽¹²⁾

3. UROGEN WELL D-ONE® KIT CONTENTS

10 Identification panels UROGEN WELL D-ONE®
10 x 10 mL Sterile physiological Saline Solution
10 Sterile Swabs

(REF. MS01281)

(REF. MS01281)

(REF. MS01304)

4.REAGENTS REQUIRED BUT NOT PROVIDED

Myco D-One Transport Medium 10 x 2 mL
Liquid Sterile Paraffin
Culture supplemented media plate, ready to use, for *Mycoplasma spp.*
General Laboratory equipment

(REF. MS01314)

(REF. MS01316)

5. PANEL COMPOSITION

Well 1: Selective Medium for the isolation of <i>Mycoplasma hominis</i>
Well 2: Selective Medium for the isolation of <i>Mycoplasma hominis</i>
Well 3: Selective Medium for presumptive identification of <i>Mycoplasma spp.</i>
Well 4: Selective Medium for presumptive identification of <i>Mycoplasma spp.</i>
Well 5: Selective Medium for growing of CCU 10^4 <i>Ureaplasma urealyticum/parvum</i>
Well 6: Selective Medium for growing of CCU $\geq 10^5$ <i>Ureaplasma urealyticum/parvum</i>
Well 7: Selective Medium for presumptive identification of <i>Ureaplasma urealyticum/parvum</i>
Well 8: Enriched Culture Medium for growth control
Well 9: Culture medium containing Levofloxacin 1 µg/mL
Well 10: Culture medium containing Josamycin 2 µg/mL
Well 11: Culture medium containing Moxifloxacin 0,25 µg/mL
Well 12: Culture medium containing Doxycycline 4 µg/mL
Well 13: Culture medium containing Levofloxacin 2 µg/mL
Well 14: Culture medium containing Josamycin 2 µg/mL
Well 15: Culture medium containing Moxifloxacin 2 µg/mL
Well 16: Culture medium containing Doxycycline 4 µg/mL
Well 17: Culture medium containing Levofloxacin 2 µg/mL
Well 18: Culture medium containing Josamycin 8 µg/mL
Well 19: Culture medium containing Moxifloxacin 0,5 µg/mL
Well 20: Culture medium containing Doxycycline 8 µg/mL
Well 21: Culture medium containing Levofloxacin 4 µg/mL
Well 22: Culture medium containing Josamycin 8 µg/mL
Well 23: Culture medium containing Moxifloxacin 4µg/mL
Well 24: Culture medium containing Doxycycline 8 µg/mL
Well 25: Selective Medium for presumptive identification of <i>Neisseria spp.</i>
Well 26: Selective Medium for presumptive identification of <i>Streptococcus agalactiae</i>
Well 27: Selective Medium for presumptive identification of <i>Staphylococcus aureus</i>
Well 28 Selective Medium for presumptive identification of <i>Enterococcus spp.</i>
Well 29: Selective Medium for presumptive identification of di <i>Escherichia coli</i>
Well 30: Selective Medium for presumptive identification of di <i>Candida spp.</i>
Well 31: Selective Medium for presumptive identification of <i>Gardnerella vaginalis</i>
Well 32: Selective Medium for presumptive identification of <i>Trichomonas vaginalis</i>

6. TEST PROCEDURE

6.1 SAMPLE COLLECTION

Sample:

endocervical exudate, vaginal exudate, urethral exudate, urine, seminal fluid

For the best performance of this system the sample should be taken aseptically according to the methodology implemented in each hospital center and before starting antimicrobial treatment.

The sample should be processed immediately after collection.

Do not refrigerate or freeze. Protect from light and sun.

The results obtained by UROGEN WELL D-ONE® should be compared with complementary methods of analysis, established in each laboratory.

6.2 PROCEDURE

Sample:

Endocervical exudate, vaginal exudate, urethral exudate:

- Take the sample according to the procedures established in each laboratory
- Resuspend the swab with the sample in sterile saline solution supplied in the kit, left in saline solution for 3-5 minutes, stirring and pressing the swab against the tube walls until obtain an homogeneous suspension.
- Add 150 µL of the obtained suspension in each well of the system panel
- Add two drops of sterile paraffin in the wells from 1 to 25.
- Incubate at 36±1 °C for 24 – 48 hours.

Urine-Seminal Liquid

- Take the sample according to the procedures established in each laboratory
- Reconstitute 200 µL (4 drops) of the obtained sample in sterile saline solution supplied in the kit
- Add 150 µL (3 drops) of final suspension obtained in each well of the system
- Add two drops of sterile paraffin in the wells from 1 to 25.
- Incubate at 36 ± 1 °C for 24 – 48 hours.

Incubation

- It is recommended to incubate the plate for 24/48 hours. It is recommended to incubate up to 6 days, for the identification of *M. spp.*

Presumptive identification of microorganisms included in the System

- *Ureaplasma spp* is positive after 18 - 24 hours. Some "Fastidious" strains of *Ureaplasma spp.* can be positive after 36 hours. The presumptive identification of *Ureaplasma urealyticum/parvum* is associated to the positivity of well 5 and 7. The positivity of well 6 depends on if the concentration (CCU/mL) of *Ureaplasma urealyticum/parvum* is $\geq 10^5$. Strains belonging to the family of *Ureaplasma spp.* are not able to growth in the well 8.
- IDENTIFICATION OF *Ureaplasma urealyticum/parvum*:

Ureaplasma urealyticum/parvum CCU 10^4

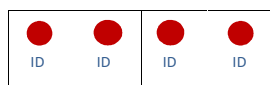
			
10 ⁴	$\geq 10^5$	ID	ID
<i>U.urealyticum/parvum</i>		GC	

Ureaplasma urealyticum/parvum CCU $\geq 10^5$

			
10 ⁴	$\geq 10^5$	ID	ID
<i>U.urealyticum/parvum</i>		GC	

- *Mycoplasma hominis*, is positive after 24/48 hours.
- *Mycoplasma spp.*, can be positive after 3 days. The incubation should be extended up to 6 days for "fastidious" species. An incubation longer than 6 days, can be realized at the discretion of the microbiologist.

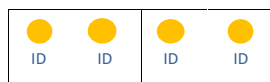
IDENTIFICATION OF *M.hominis*:



M.hominis

M.spp

IDENTIFICATION OF *M.spp.*:



M.hominis

M.spp

Some strains of *M.spp.*, change the colour of the identification wells in different time. The positivity of at least one well of identification in *M.spp* area, after 3 days of incubation is an indicator of positivity.

- The antimicrobial susceptibility wells area are intended for *M. hominis* and *Ureaplasma urealyticum/parvum*. The species of *M.spp* do not cause the colour changing in this area.
- The presence of a transparent yellow color in ID wells for *M.spp.*, should be confirmed by complementary techniques to detect mycoplasmas. In order to perform this confirmation, it is necessary to take carefully the content of the well.
- In the case that a turbidity is observed in the well 25 *Neisseria spp.*, it is recommended to perform an oxidase test; the confirmation of the *Neisseria spp.* is realized sowing the well content in a selective medium.
- It is recommended to perform the test of Sodium Hippurate on the content of well 26 *Streptococcus agalactiae*. Just strains of *S. agalactiae* non haemolytic can take up to 48 hours for the colorimetric reaction in the well, or cause any kind of turbidity without colour changing. The Sodium Hippurate test in disk or tube, as well as the latex test of the well content accelerate the microbial identification.
- It is recommended to perform from the well 27 *Staphylococcus aureus* a confirmatory coagulase tube test or a latex test, despite of the 99,9% of strains of *S.aureus* are positive to the medium contained in the system.
- The positivity of the well 28 is associated to the presence of *Enterococcus spp.*
- The medium included in the presumptive identification well of *E.coli*, detect *E.coli* inactive.
- In the case of well 30 and 32, it is recommended a microscopic observation (40 X) of culture media containing in the identification well. Observe the morphological features of *Candida spp.* and *Trichomonas vaginalis*.
- In the case of the well 31, a red colour is an indicator of bacterial growth. It is recommended a microscopic observation (100 X) to notice the morphological features of *Gardnerella vaginalis*.

Incubations longer then 24-48 hours may be carried out based on the consideration of the microbiologist, for a period of 5 days, but the formulations of this kit allow the growth of the most common urogenital mycoplasma (*Mycoplasma hominis* and *Ureaplasma urealyticum / parvum*) in 18 - 48 hours ⁽⁵⁻⁶⁾.

In the case of incubations longer than 48 hours, it may occur a volume decrease of well 30 and 31, that are indicated for a possible microscopic observation of the microbial structures. The reading of results, despite of this, is not compromised. Nevertheless, to perform the microscopic observation, it can be added a drop of physiological saline solution in order to resuspend the content before preparing the slide. The colorimetric reaction in the well does not interfere with the observation of the microbial structure.

It is recommended to use of traditional methods established for the conventional identification of microorganisms that can be detected with this test ⁽⁵⁾.

7. RESULTS INTERPRETATION

The results are evidenced by color changes that occur in wells according to reactions due to chemical or chromogenic components contained in specific formulations for each microorganism (See The Reaction Table).

Additional microscopic observation is recommended as described in this IFU.

Culture of samples is recommended in selective media mentioned in section 4 (Materials required but not provided) in the case of positive samples that have to be confirmed. To perform this procedure, the samples can be stored in the transport medium referred in paragraph 4 or it can be realized a culture from the Wells ID System.

8. WARNING AND PRECAUTIONS

1. For professional and *in vitro* diagnostic use only, not to be used by the general public.
2. The samples have to be treated as potentially infectious and the test must be carried out only by trained personnel handling clinical and / or microbiological laboratory techniques.
3. Do not open the sealed pouch unless ready to perform the assay.
4. Do not use expired devices.
5. Do not use components from any other type of test kit as a substitute for the components in this kit.
6. Wear protective clothing and disposable gloves while handling the kit reagents and clinical specimens. Wash hands thoroughly after performing the test.
7. Do not smoke, drink or eat in areas where specimens or kit reagents are being handled.
8. Dispose of all specimens and materials used to perform the test as bio-hazardous waste

9. LIMITATIONS

Samples collected after antimicrobial treatment may provide false - negative results⁽⁴⁻⁵⁾.

The samples such as vaginal or endocervical exudate, with pH values due to infrequent particular infectious processes can cause nonspecific reactions with positive colorations in the identification and antimicrobial wells for urogenital mycoplasmas.⁽⁷⁻⁸⁾

A sample with a high concentration of color changing units of urogenital mycoplasmas can lead to widespread positivity of identification panel, in such cases it is recommended to dilute the sample⁽⁷⁻⁸⁻⁹⁾.

The use of the special Myco D-One Transport Medium for sample is recommended for the preservation of samples and / or transportation. In this way samples can be stored at 4 °C for 72 hours or frozen at -70 °C for 8 weeks.

A collection or an improper storage may cause sample microbiological contamination or incompatible agents with the proper functioning of this kit.

The presence of microorganisms responsible of urease enzyme, causes a reaction in the well 8.

The presence of a high concentration of Gram negative bacteria, *Streptococcus spp.*, *Enterococcus spp.*, *S.aureus*, can causes a colour changing in mycoplasmas area, in this cases a turbidity is observed in wells, in addition to the colour changing and the positivity of the relative identification wells.

The possible presence of *Pseudomonas spp.* or multi resistant microorganisms cause nonspecific reactions in the system.

Read this instructions for use carefully, in order to avoid mistakes

10. QUALITY CONTROL

Each batch of UROGEN Well D-ONE is submitted to a rigorous quality control with the following bacterial reference strains. The control strains are used for both positive reactions from different wells to check the proper functioning of the media formulations of the different wells before any nonspecific reactions.

“non fastidious” Mycoplasmas

Strain	ATCC	
<i>Mycoplasma hominis</i>	23114	
<i>Mycoplasma penetrans</i>	55252	
<i>Ureaplasma urealyticum</i>	27618	For growth control
<i>Ureaplasma urealyticum</i>	33175	To detect the antimicrobial susceptibility
<i>Ureaplasma parvum</i>	27815	

“Fastidious” Mycoplasmas o “Slow growing Mycoplasmas”

Strain	ATCC
<i>Mycoplasma genitalium</i>	33175
<i>Mycoplasma fermentans</i>	19989
<i>Mycoplasma hyorhinis</i> *	29052

*The inclusion of strain *Mycoplasma hyorhinis* (cultivar alpha) was performed to test the ability of the formulation to promote the growth of difficult species (*M. genitalium* and *M. fermentans*) in traditional culture media because the dynamics of growth and nutritional requirements of these species are very similar.

Other tested strains

Candida albicans ATCC 10231
Candida tropicalis ATCC 13803
Candida krusei ATCC 14243
Gardnerella vaginalis ATCC 14019
Trichomonas vaginalis ATCC 30001
Escherichia coli ATCC 25922
Neisseria gonorrhoeae ATCC 19424
Streptococcus agalactiae ATCC 13813
Staphylococcus aureus ATCC 25923
Streptococcus faecalis ATCC 19433

The strains used for Quality Control have been carefully selected according to the recommendations published for the growth and susceptibility to antimicrobial agents⁽⁵⁻¹⁰⁾. Each laboratory should establish its own internal quality controls.

11. STORAGE

Store at 2-8 °C in its original package. Do not store near heat sources and avoid extreme temperature variations. Under these conditions the product is valid until the expiration date shown on label of primary and secondary box. Do not use after this date. Discard if there are signs of deterioration.

12. BIBLIOGRAPHY

1. Waites KB, Schelonka RL, Xiao L, Grigsby PL, Novy MJ. Congenital and opportunistic infections: *Ureaplasma* species and *Mycoplasma hominis*. *Semin Fetal Neonatal Med.* 2009;13(4):190–9
2. Kallapur SG, Kramer BW, Jobe AH. *Ureaplasma* and BPD *Semin Perinatol.* 2013;37(2):94-101
3. WHO Interim Guidelines 2007: Infection prevention and control of epidemic- and pandemic-prone acute respiratory diseases in health care. WHO/CDS/EPR/2007.6
4. Begoña JCC, Meseguer MAP, Oliver APP, Puig de la Bellacasa J Diagnóstico microbiológico de las infecciones bacterianas del tracto respiratorio inferior. *Procedimientos en Microbiología Clínica.* SEIM 2007. Ed. Cercenado E y Cantón R
5. Acosta BB, Gema MC, Matas GL. Diagnóstico microbiológico de las infecciones por *Mycoplasma* spp. y *Ureaplasma* spp. 2007 Ed. Cercenado E y Cantón R. SEIM PNT-M-03. Procesamiento microbiológico de

las muestras genitourinarias y de otras procedencias para cultivo de *Ureaplasma* spp. y *Mycoplasma hominis*

6. Validazione del Sistema UROGEN WELL D-ONE® in campioni da Infezioni da *Mycoplasma* spp. ed altri patogeni urogenitali. Comparazione con metodi tradizionali. UROGEN WELL D-ONE® Technical File, C.P.M. sas, 2013.

7. Krauss-Silva L, Almada-Horta A, Alves MB, Camacho KG, Moreira ME, Braga A. Basic vaginal pH, bacterial vaginosis and aerobic vaginitis: prevalence in early pregnancy and risk of spontaneous preterm delivery, a prospective study in a low socioeconomic and multiethnic South American population. BMC Pregnancy Childbirth. 2014 ;14:107. doi: 10.1186/1471-2393-14-107

8. Hemalatha R, Ramalaxmi BA, Swetha E, Balakrishna N, Mastromarino P. Evaluation of vaginal pH for detection of bacterial vaginosis Indian J Med Res. 2013 ;138(3):354-9.

9. Granato PA, FAAM D. Vaginitis: Clinical and Laboratory Aspects for Diagnosis. Clin Microb New. 2010; 32(15):111-6.

10. Clinical and Laboratory Standard Institute (CLSI), M43-A, Methods for Antimicrobial Susceptibility Testing for Human Mycoplasmas; Approved Guideline, First Edition (2011)

11. Favalli C, Favaro M, Diagnosis of Mycoplasma and Ureaplasma infection by a New Accelerated Micro-Method. ECCMID, 2015

12. Favalli C, Favaro M, Comparative study of a new system MYCO WELL D-ONE with molecular methods. Università di Tor Vergata, 2014

	COLORIMETRIC REACTIONS	
	POSITIVE	NEGATIVE
Well 1: Selective Medium for the isolation of <i>Mycoplasma hominis</i>	RED	YELLOW
Well 2: Selective Medium for the isolation of <i>Mycoplasma hominis</i>	RED	YELLOW
Well 3: Selective medium for the presumptive identification of <i>Mycoplasma spp.</i>	YELLOW	RED
Well 4: Selective Medium for the presumptive identification of <i>Mycoplasma spp.</i>	YELLOW	RED
Well 5: Selective Medium for the growth of CCU 10 ⁴ <i>Ureaplasma urealyticum/parvum</i>	RED	YELLOW
Well 6: Selective Medium for the growth of CCU ≥ 10 ⁵ <i>Ureaplasma urealyticum/parvum</i>	RED	YELLOW
Well 7: Selective Medium for the presumptive identification of <i>Ureaplasma urealyticum/parvum</i>	RED	YELLOW
Well 8: Culture Medium to test the presence, in the sample, of microorganism responsible for urease enzyme that do not belong to the family of <i>Ureaplasma urealyticum</i> (Strain s of <i>Ureaplasma spp.</i> , are not able to grow in the well 8)	YELLOW	RED
	ANTIMICROBIAL SUSCEPTIBILITY TEST	
	RESISTANT	SENSIBILE
Well 9: Culture medium containing Levofloxacin 1 µg/mL	RED	YELLOW
Well 10: Culture medium containing Josamycin 2 µg/mL	RED	YELLOW
Well 11: Culture medium containing Moxifloxacin 0,25 µg/mL	RED	YELLOW
Well 12: Culture medium containing Doxycycline 4 µg/mL	RED	YELLOW
Well 13: Culture medium containing Levofloxacin 2 µg/mL	RED	YELLOW
Well 14: Culture medium containing Josamycin 2 µg/mL	RED	YELLOW
Well 15: Culture medium containing Moxifloxacin 2 µg/mL	RED	YELLOW
Well 16: Culture medium containing Doxycycline 4 µg/mL	RED	YELLOW
Well 17: Culture medium containing Levofloxacin 2 µg/mL	RED	YELLOW
Well 18: Culture medium containing Josamycin 8 µg/mL	RED	YELLOW
Well 19: Culture medium containing Moxifloxacin 0,5 µg/mL	RED	YELLOW
Well 20: Culture medium containing Doxycycline 8 µg/mL	RED	YELLOW
Well 21: Culture medium containing Levofloxacin 4 µg/mL	RED	YELLOW
Well 22: Culture medium containing Josamycin 8 µg/mL	RED	YELLOW
Well 23: Culture medium containing Moxifloxacin 4 µg/mL	RED	YELLOW
Well 24: Culture medium containing Doxycycline 8 µg/mL	RED	YELLOW
	POSITIVE	
	POSITIVE	NEGATIVE
Well 25: Selective Medium for the presumptive identification of <i>Neisseria spp.</i>	CLOUDY YELLOW	YELLOW
Well 26: Selective Medium for the identification of <i>Streptococcus agalactiae</i>	GREEN	LIGHT YELLOW
Well 27: Selective Medium for the presumptive identification of <i>Staphylococcus aureus</i>	DARK PURPLE	LIGHT YELLOW
Well 28: Selective Medium for the presumptive identification of <i>Enterococcus spp.</i>	BLACK	LIGHT YELLOW
Well 29: Selective Medium for the presumptive identification of <i>Escherichia coli</i>	GREEN	WHITE
Well 30: Selective Medium for the presumptive identification of <i>Candida spp.</i>	GREEN	NO COLOUR /CLOUDY WHITE
Well 31: Selective Medium for the presumptive identification of <i>Gardnerella vaginalis</i>	RED	NO COLOUR
Well 32: Selective Medium for the presumptive identification of <i>Trichomonas vaginalis</i>	LIGHT BLUE	LIGHT BLUE

Index of Symbols

 Reference code  LOT number  Expiration date

 Temperature limitation/Store between  For *in vitro* diagnostic use

 Do not reuse  Fragile, handle with care  Consult Instructions for Use

 Manufacturer  Contains sufficient for <n> tests

CE

Conform to the Directive 98/79/EC on *in vitro* diagnostic medical device



C.P.M. sas – Via degli Olmetti, 5 – 00060 (Zona Ind.le) Formello, (ROMA), Italia

Tel. +39/06.90.400.126 +39/06.90.75.531 - Fax. +39/06.90.405.270 –

www.cpmsas.it - cpm@cpmsas.it

